Patient With Large 17p11.2 Deletion Presenting With Smith-Magenis Syndrome and Joubert Syndrome Phenotype

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We report on a 22-year-old woman carrying a del(17)(p11.2p12) and presenting with the clinical manifestations of both Smith-Magenis syndrome (SMS) and Joubert syndrome (JS). Her facial anomalies, brachydactyly, severe mental retardation, and self-injuring behavior could be attributed to SMS, whereas the cerebellar vermis hypoplasia, hypotonia, ataxic gait, developmental delay, and abnormal respiratory pattern were suggestive of JS. By fluorescent in situ hybridization analyses with Yeast Artificial Chromosomes (YAC) mapping to the 17p11.2 region, as well as locus-specific probes generated through a novel procedure, we could establish that the deletion encompasses a 4-Mb interval with centromeric and telomeric breakpoints at loci D17S793 and D17S953, the latter close to the locus Charcot Marie Tooth 1A (CMT1A)-REP. The deletion differs from that commonly found in SMS in its telomeric boundary, which is more distal than usually observed. The presence of JS phenotype in our patient and the detection of an unusual SMS deletion might suggest the presence of a JS gene in close proximity to the SMS locus.

INTRODUCTION

Joubert syndrome (JS) (OMIM #213300) was first described by Joubert et al. in 1969. It is presumed to have an autosomal recessive pattern of inheritance because of the number of affected sibs and consanguineous parents.

The diagnostic criteria of JS provided by Saraiva and Baraitser [1992], include cerebellar vermis hypoplasia, hypotonia, developmental delay, and at least one of two additional anomalies (abnormal breathing and abnormal eye movements). Chorioretinal coloboma, retinal dystrophy, renal cysts, polydactyly, and occipital myelomeningocele may also be associated with JS. To date no clues to chromosomal location and to the molecular basis of JS are yet available.

Smith-Magenis syndrome (SMS) (OMIM #182290) is a well-defined contiguous gene syndrome characterized by multiple congenital anomalies and mental retardation. Since the first report by Smith et al. in 1982, more than 100 cases have been described [Juval et al., 1996]. The incidence of SMS has been estimated to be approximately 1/25,000 births [Smith et al., 1998], although this is likely to be an underestimate. The clinical phenotype of SMS includes peculiar facial anomalies, brachydactyly, short stature, a variable degree of developmental delay, signs of neuropathy, and neurobehavioral problems, including sleep disturbances and self-injurious behavior [Allanson et al., 1999].

An interstitial deletion on the short arm of chromosome 17, involving the 17p11.2 region, is the cytogenetic marker in all SMS patients.

Despite the clinical variability of SMS, which may also be related to the size of the deletion, patients sharing both SMS and JS stigmata have never been reported. Cerebral anomalies have been described in a few SMS patients, but the involvement of the cerebellum has been reported in only one case who, to the best of our knowledge, did not show any other JS symptoms [Greenberg et al., 1996]. We report on a 22-year-old female carrying a del(17)(p11.2p12) and presenting with the clinical manifestations of both SMS and JS.

CLINICAL REPORT

The 25-year-old female patient was born to a healthy, non-consanguineous couple. Two brothers are

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healthy. Pregnancy was complicated by bleeding during the first trimester. Delivery was at 32 weeks of gestation with a birth weight of 2 kg (50th centile). From early infancy until puberty, the patient’s weight, height, and occipitofrontal head circumference (OFC) were always below the normal standards. From early on she had crises of abnormal breathing pattern (hyperpnea and apnea) and developed seizures after age 5 years.

Upon clinical examination at age 22 years, her height was 143 cm (<3rd centile), weight 46 kg (<3rd centile), OFC 51 cm (<3rd centile), inner canthal distance 3.5 cm (>97th centile), and outer canthal distance 10.8 cm. The lengths of her hand and middle finger were 16.5 cm (3rd–25th centile) and 6.5 cm (<3rd centile), respectively.

The patient presented with microcephaly, brachycephaly, hypertelorism, depressed nasal bridge, malar hypoplasia, short philtrum, prominent mandible, small and posteriorly angulated ears (Fig. 1A), as well as brachydactyly of hands and feet. She had inguinal, axillary, right thoracal and abdominal freckling, and five skin café-au-lait spots. Neuropsychological examination showed an ataxic gait, generalized hypotonia, a
right lesion of the ulnar nerve due to a mild trauma, severe language delay, profound mental retardation, self-injuring behavior, abnormal breathing pattern (hyperpnea and apnea) and seizures. Brain nuclear magnetic resonance (NMR) showed cerebellar vermis hypoplasia, dilated cerebral ventricles, subarachnoid spaces (Fig. 1B), and focal signals of alterations in the thalamus and basal nuclei, probably related to a perinatal damage. The presence of bilateral retinal lacunae was demonstrated by ophthalmological examination at age 18 years. Later examinations showed detachment of the lower retinal segment with extensive vitreoretinal proliferation.

**CYTOGENETIC AND MOLECULAR STUDIES**

Chromosome spreads from the proposita and her parents were obtained from phytohemagglutinin (PHA)-stimulated peripheral blood lymphocytes, and G-banded using Wright’s stain; the karyotype has been described according to the International System for Human Cytogenetic Nomenclature (ISCN 1995).

Genomic DNA was extracted using standard procedures. The polymerase chain reaction (PCR) assays involved the use of primers for D17S921, D17S1857, D17S953, D17S71, D17S1871, D17S959, and D17S842 loci. All of the primer sequences can be found in the Genome Data Base (http://www.gdb.org).

For all markers, one primer was labeled with $[\gamma^33\text{P}]\text{ATP}$ (Amersham, Buckinghamshire, UK), and the PCR products were separated on 6% polyacrylamide gels.

Locus-specific probes were also prepared as described in detail by Riva et al. [1998]. The method is based on two consecutive PCR reactions using YAC clones containing the loci of interest. Probes specific for D17S793 and D17S8953 loci were generated from YACs 828b9 and 976g4 using specific primers (given in the Genome Data Base), and polymerized by subjecting single-strand DNA to combined locus-specific and Alu PCR. The Alu PCR products were then collected, precipitated, and labeled for fluorescent in situ hybridization (FISH) analysis.

The FISH experiments were performed according to standard procedures [Montanaro et al., 1991]. The probes were labeled with digoxigenin-dUTP (Boehringer Mannheim, Germany) using a nick translation kit (Boehringer Mannheim, Germany).

The chromosomes were counterstained with propidium iodide 0.6 μg/mL in antifade (Oncor), and then visualized using a Leitz DM-RB microscope equipped for 4’,6-diamino-phenylindole and fluorescein isothiocyanate/tetramethyl rhodamine isothiocyanate (TRITC) epifluorescence optics. The images were captured by means of a charge coupled device (CCD) camera (Hamamatsu 3CCD Camera, C5810) and visualized using Highfish software (Casti Imaging, Venice, Italy).

**RESULTS**

The cytogenetic analysis of the proposita showed a del(17)(p11.2p12) in all of the analyzed metaphases (Fig. 2a). The parents had normal chromosomes. This finding, combined with a SMS phenotype and clinical signs such as cerebellar vermis hypoplasia, hypotonia, developmental delay, and an abnormal respiratory pattern characteristic of JS, suggested the presence of a contiguous gene syndrome combining SMS and JS phenotype. Table I reports the proposita’s clinical findings in comparison with those of SMS and JS patients.

To confirm the involvement of the SMS region in the cytogenetic deletion, we performed FISH analyses using the SMS probe (Oncor), which gave only one signal on one chromosome 17 (Fig. 2b). To define the boundaries of the deletion, the patient and her parents were tested for seven short-tandem-repeat polymorphic markers of the 17p11.2-12 region.

Short-tandem-repeat polymorphic analysis for D17S1824, D17S959, and D17S1871 (listed cen to tel) demonstrated heterozygosity. Four markers (D17S71,
D17S1857, D17S953, and D17S921) showed only one allele in the patient, but the parental allele pattern was uninformative (data not shown). On the basis of the segregation analysis, a deletion with D17S1871 as centromeric boundary was suspected. To confirm this view and define more precisely the boundaries of the deletion, contiguous YACs [Chen et al., 1997] were used for FISH analysis on metaphases from the patient. FISH with 719c6, 795c9 and 951b11 YACs gave a signal only on one chromosome 17 in all of the analyzed metaphases (Fig. 3, b and c), indicating that the deletion encompasses the SM region and is comprised between the CMT and the SMS region. FISH with YAC 828h9 showed a decreased signal on the deleted chromosome in comparison with the normal homologue (Fig. 3d). YACs 961f10 and 976g4, encompassing the CMT region showed signals on both homologues in all of the analyzed metaphases, ruling out the involvement of the CMT region (data not shown). FISH experiments with D17S793 (spanning the proximal CMT-REP) and D17S953 (within the SM chromosomal region) probes showed hybridization signals on both homologues (Fig. 3, a and e), thus fixing the deletion’s boundaries to these loci. On the basis of the minimum tiling path of the contig YACs [Chen et al., 1997], the size of the deletion was estimated to be at least 4 Mb.

**DISCUSSION**

JS is a rare condition with variable clinical signs, whose diagnostic criteria have been defined by Saraiva and Baraitser [1992]. Recently, Maria et al. [1999] described the presence of a complex malformation of the brainstem and cerebellum in JS patients. The diagnosis of JS in our proposita fulfills the above mentioned diagnostic criteria, although cerebellar vermis hypoplasia is the only neuroradiological finding.

The molecular characterization of the 17p11.2p12 deletion confirmed that the deletion: 1) involved the SMS region; 2) spanned at least 4 Mb; and 3) had boundaries that were somewhat displaced in comparison with those commonly observed in SMS patients (Fig. 3).

The genetic anomaly marking SMS patients is well described; on the basis of molecular analysis, the most common SMS deletion is approximately 5 Mb [Lupski, 1998], but smaller and larger deletions ranging from 2 to 9 Mb have also been reported [Smith et al., 1998]. It has been demonstrated that the SMS common deletions are mediated by a recombination mechanism between repeat gene clusters (SMS-REPP and SMS-REPD) localized 5 Mb apart in 17p11.2 [Chen et al., 1997]. A few patients with distinct deletions have been reported [Juval et al., 1996; Greenberg et al., 1991], and the underlying mechanism seems to be due to the high number of repeated sequences in the SMS chromosomal region (SMCR) apart from SMS-REPs [Juval et al., 1996]. The coincidence of the boundaries of the atypical deletion observed in our patient with repeat sequences of the same family (Fig. 3) is in agreement with this view.

The deletion, which was more accurately characterized by FISH analysis with locus-specific probes, extends from D17S793 to D17S953 STSs, which flank the two repeat clusters.

Similar deletions extending as far as D17S953 have been described in four of 70 SMS patients [Juval et al., 1996; Greenberg et al., 1991], but none of them was reported as having JS symptoms.

The chromosomal location and molecular basis of JS are still unknown. Pellegrino et al. [1997] screened a panel of 18 JS patients for mutations in Wingless-Type mmvt Integration Site Family Member 1 gene (WNT1) located in 12q12-q13, on the basis of its expression in the developing cerebellum and the associated mutations in a JS animal model, but no mutations were detected.

Recently Saar et al. [1999] performed a whole genome scan in two consanguineous families with multiple JS-affected probands. The authors evidenced in one family linkage with chromosome 9q34.3, that was excluded for the other family, suggesting genetic heterogeneity.

The presence of JS in our patient, together with the detection of an unusual SMS deletion, suggests that the interval between D17S793 and D17S953 may harbor a candidate JS gene.

Since JS is a recessive condition, it can be hypothesized that a mutated JS allele on chromosome 17p is unmasked by the loss of the wild-type allele on the 17p11.2p12 deleted chromosome.

Of the 16 genes so far mapped within SMCR, one is responsible for the autosomal recessive Sjogren-Larsson syndrome (SLS) [De Laurenzi et al., 1996] and another one for a non-syndromal recessive form of deafness (DFBN3) [Liang et al., 1998]. Although there have not yet been any reports of patients with both SMS and SLS, it has to be underlined that the rarity of the condition may mean that SLS carrier frequency is very low, thus making the detection of both SMS and SLS highly unlikely.

Regarding the non-syndromal form of deafness, there is so far no evidence to ascribe the deafness, re-

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### TABLE I. Clinical Findings of the Present Case: Cross Evaluation With SMS and JS Manifestations

<table>
<thead>
<tr>
<th>Present case</th>
<th>SMS</th>
<th>JS</th>
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<tbody>
<tr>
<td>Bachycephaly</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Broad face</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Telecanthus</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Midface hypoplasia</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Broad nasal bridge</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Short philtrum</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Prominent mandible</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Brachydactyly</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Signs of neuropathy</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Severe language delay</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Self-injuring behavior</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Seizures</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Cerebellar vermis hypoplasia</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Hypotonia</td>
<td>−</td>
<td>+</td>
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<tr>
<td>Ataxic gait</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Abnormal breathing pattern</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>CALs and freckling</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

+ Presence of clinical sign; −, absence of clinical sign; CALs, cafe-au-lait spots.
Fig.3. **Left panel:** Schematic representation of 17p11.2-p12 (modified from Chen et al. [1997]). The boxes with vertical and horizontal lines represent two distinct families of repeated sequences: the black boxes represent CMT1A-REPs, and the white boxes the SMS REPs, comprising distal (D), median (M), and proximal (P) REPs. The filled bars flanking the map to the right indicate sequentially the CMT1A and SMS chromosomal regions (red), respectively, the patient’s deletion (green), and the YAC clones from a contig covering this region (black); distances are not to scale. The right panel shows representative FISH results. The two locus-specific probes 953 and 793 give two fluorescent signals on chromosomes 17p (arrowheads), thus marking the deletion boundaries (a and e); YACs 719c6 and 795c9 hybridize only to one chromosome 17p (indicated by the arrowhead; the deleted 17p is marked with an arrow) thus mapping within the deletion (b and c); YAC 828b9 gives a decreased signal on one chromosome 17p, which indicates that it spans the centromeric boundary of the deletion (d).
ported in a few SMS patients, to the DFBN3 syndrome. On the other hand, the estimated carrier frequency of 1/259 for JS [Pellegrino et al., 1997] could justify the coincidence of JS and SMS in the same patient with del(17p). However, in order to prove the localization of the JS locus within the 17p interval comprised between D17S953 and D17S793, a targeted 17p11.2 linkage analysis needs to be performed in suitable JS families.

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REFERENCES


